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BIOCHEMICAL MECHANISMS IN INFLAMMATION*

BY

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Inflammation is a manifestation of severe cell injury in vertebrates. Any agent that interrupts normal metabolic processes may induce an inflammatory reaction. The irritant may be of exogenous or of endogenous origin. It may be physical in nature, chemical, or viable, as in the case of a micro-organism. The sequential pattern of response is quite stereotyped. Variations are attributed either to the specific chemistry of the irritant or to the anatomical location of the lesion. Here I can give only a broad panorama of our point of view based on experimental observations gathered for thirty years. I regret that limit on space prevents me from discussing the important contributions of other investigators.

In the first place an inflammatory reaction is initiated by a disturbance in local fluid exchange (Menkin, 1940a). There is a preliminary vasoconstriction followed by a vasodilatation, and, most important of all, an increase in capillary permeability. This latter phenomenon was brought first to serious attention by Cohnheim (1889). The extent of this increased permeability is evidenced by the passage of graphite particles or of bacteria through the endothelial wall at the site of an acute inflammation (Menkin, 1931a). It is of significance to determine the mechanism of this increased capillary permeability in an inflamed area. The last word on this mechanism, as on everything else in biology, has not yet been fully settled. In 1936 it was observed that an inflammatory exudate *per se* induces an increase in capillary permeability (Menkin, 1936). There seems to exist a permeability factor in the exudate, which in turn is considered to represent the products of cell injury admixed with elements from the circulating blood. Blood serum, however, is incapable of inducing such a reaction or at least an identical pattern of response. The permeability factor present in exudates can be precipitated with 20% sodium sulphate or at half saturation with ammonium sulphate (Menkin, 1940a).

Leucotaxine

By preliminary deproteinization with pyridine or dioxan and acetone, the permeability factor was purified. Subsequently it was treated with butyl alcohol or N acetic acid (Menkin, 1938a, 1940a, 1953a) (Fig. 1). The purified product consisted of doubly refractile granules in an ill-defined matrix (Menkin, 1938a). This could at times be brought to the crystalline state as needle-like crystals, though this procedure is not always successful (Menkin, 1938a, 1940a). In addition, this substance induced first the close adherence of polymorphonuclear leucocytes to the endothelial wall, as well as their subsequent migration or diapedesis into the extracapillary spaces (Fig. 2). This migratory activity failed to occur when blood serum was extracted by the same scheme. This substance is diffusible from the whole exudate and

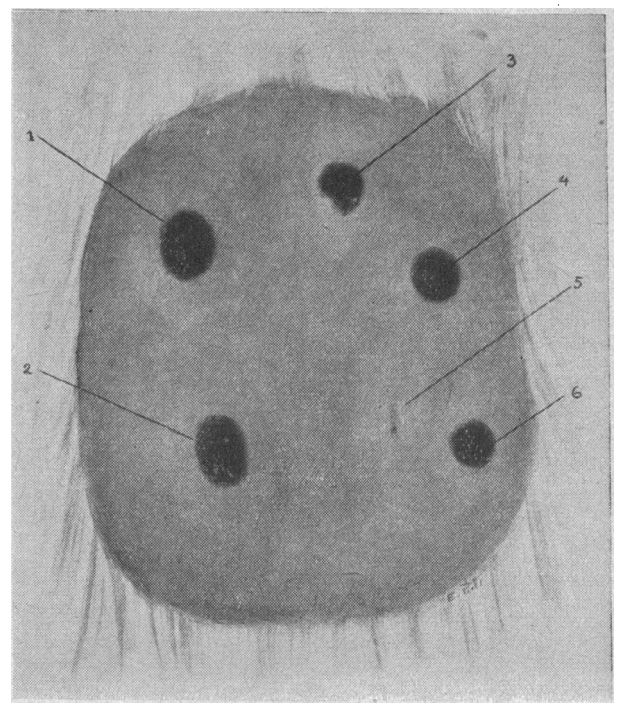

FIG. 1.—Drawing illustrating the effect of several leucotaxine fractions in increasing capillary permeability in a rabbit: *Areas 1 and 2:* Injected intracutaneously 5 mg. of fraction N in saline (for isolation cf. Menkin, 1940a, Chapter IV, p. 34). About 42 to 43 minutes after local injections of this fraction, there is marked accumulation of trypan blue from the circulating bloodstream. *Area 3:* This represents the accumulation of trypan blue owing to an increased capillary permeability 43 minutes after injection of a C–D fraction (Menkin, 1940a, p. 34). *Area 4:* Effect on permeability of capillaries produced by the local injection of a C fraction of leucotaxine (Menkin, 1940a, p. 34). The leucotaxine fraction was injected 40 minutes earlier. *Area 5:* The cutaneous injection of 0.5 ml. of saline fails to alter capillary permeability as indicated by the absence of any dye accumulating from the circulating blood 40 minutes after such an injection. *Area 6:* A C–D fraction of leucotaxine extracted from an exudate of a human being with peritonitis. Thirty-eight minutes after its cutaneous injection the dye is seen to have conspicuously localized in the treated cutaneous area.

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it is thermostable (Menkin, 1938a, 1940a). It displays two of the important biological properties of inflammation—namely, the ability of increasing capillary permeability and of inducing the migration of leucocytes (Menkin, 1938a, 1938b). It has been termed "leucotaxine" (Menkin, 1938a, 1938b).

The chemotactic property elicited by leucotaxine can be demonstrated by *in vitro* studies (Menkin, 1940a, 1955, 1956). The placing of a particle of leucotaxine on a supravitally stained smear containing polymorphonuclears from an exudate is followed by an orientation and aggregation of these cells around the particle of leucotaxine (Menkin, 1955, 1956). As controls, neither carbon particles nor iron powder are capable of inducing any such chemotactic response (Menkin, 1940a, 1955, 1956). Recently we have found that an exudate composed primarily of mononuclear phagocytes as encountered in the later or acid stage of an acute inflammation, when in contact *in vitro* with leucotaxine, likewise exhibits within a period of hours a peripheral aggregation of these phagocytes (Menkin, 1956). It is therefore conceivable that leucotaxine is perhaps also chemotactic for the mononuclear phagocytes. These cells, however, as shown by Harris (1954), are slower than the polymorphonuclears in their mobility towards an identical target. It is therefore possible that the peripheral spatial location of these cells in an inflamed area is also referable in part to leucotaxine (Menkin, 1956). Further *in vivo* observations are necessary in order to substantiate this view.

Leucotaxine appears to be a polypeptide (Menkin, 1938a, 1940a). Inactivation of its biological properties with aminopeptidase supports this view established earlier by other tests (Menkin, 1938a, 1956) (Figs. 3 and 4). Recent paper chromatographic studies by my former associate, Dr. W. Kalnins, further substantiate this view (Menkin, 1956). Whether there is a prothetic group attached to leucotaxine remains yet to be determined. A lipoidal group was considered. Lipase fails to inhibit leucotaxine. The activity appears to reside in the peptide linkages. In brief, this substance is biologically specific. It has been purified to some extent, but considerable studies are still required in order

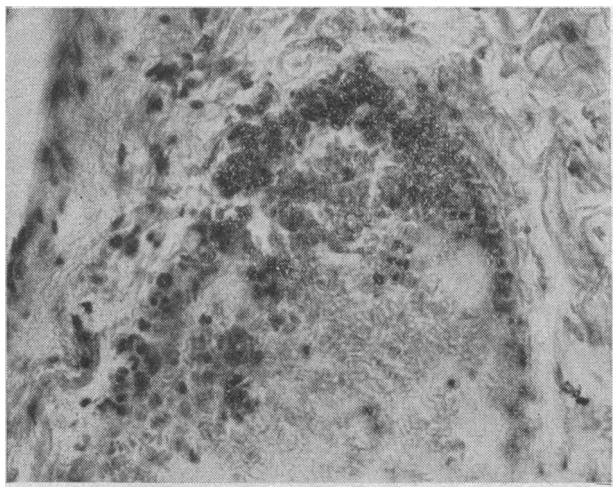

FIG. 2.—Leucotaxine, 113 μg./ml., induces not only increased capillary permeability, but likewise margination with subsequent diapedesis of polymorphonuclear leucocytes through the walls of a small venule. (×285.)

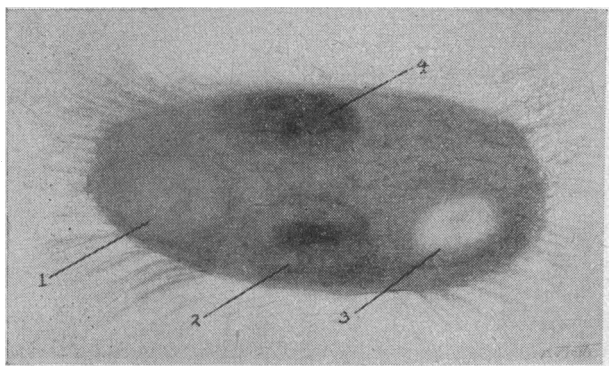

FIG. 3.—Drawing to show the effect of aminopolypeptidase in inactivating leucotaxine: *Area 1:* 2 ml. of aminopolypeptidase in 1 ml. of phosphate buffer at *p*H 8.0 incubated with 5 mg. of C–D leucotaxine fraction inactivates the ability of leucotaxine to increase capillary permeability as gauged by the failure of trypan blue to accumulate from the circulating blood into the treated cutaneous area. *Area 2:* 5 mg. of C–D leucotaxine incubated in 3 ml. of buffer at *p*H 8.0 fails to alter the capacity of leucotaxine from increasing capillary permeability as indicated by the local accumulation of trypan blue. *Area 3:* Aminopolypeptidase in 1 ml. of buffer at *p*H 8.0 induces a blanched area, and therefore, if anything, the enzyme induces only a contractility of small vessels with no evidence of any seepage of dye from the circulating blood. *Area 4:* The C–D leucotaxine fraction used above but taken up in 3 ml. of saline induces marked increase in capillary permeability as indicated by the conspicuous staining of the local cutaneous area which follows the seepage of dye from the circulating blood. The observations support the view that leucotaxine is a polypeptide which is readily inactivated by an aminopolypeptidase.

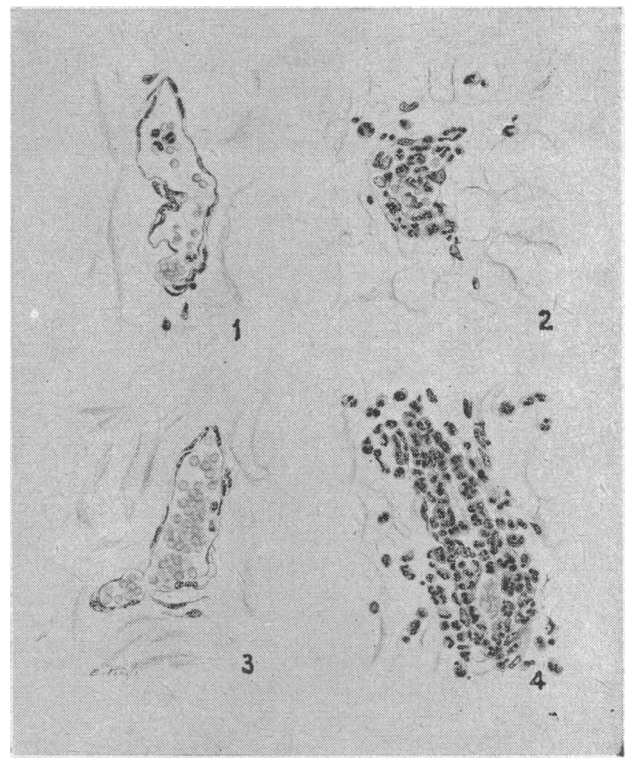

FIG. 4.—Drawing showing the inactivation of leucotaxine by both acid and enzymatic hydrolysis. *Area 1:* A capillary vessel in a cutaneous area injected with leucotaxine first hydrolysed by 50% HCl. The procedure has inactivated the ability of leucotaxine to induce leucocytic migration. *Area 2:* A capillary vessel in a cutaneous area treated with leucotaxine. Note, in contrast to the effect of acid hydrolysis in Area 1, the prominent number of polymorphonuclear leucocytes in the lumen as they are migrating outward. *Area 3:* When leucotaxine is inactivated by an enzyme such as aminopolypeptidase, there follows a conspicuous absence of any migratory activity on the part of polymorphonuclear leucocytes. *Area 4:* In contrast to Area 3, the same untreated fraction of leucotaxine induces marked chemotactic activity as indicated by conspicuous margination and diapedesis of leucocytes. The magnification utilized was about ×600.

to obtain complete purity, as well as additional chemical information concerning the precise structure of this significant biological substance. Since leucotaxine has been shown (Menkin, 1956) to be chemotactic both *in vivo* and *in vitro*, the objections recently raised again by Harris, without bringing any factual experimental data, appear to be without foundation (Harris, 1960). Similar arguments have already been presented in the past, and have been adequately treated by me (Harris, 1954, Menkin, 1955, 1956). Harris, however, in his recent review ignores these replies (Harris, 1960).

In an endeavour to throw light on the precursors in the injured cell of leucotaxine, the method of differential centrifugation was employed (Menkin, 1958a). Leucotaxine was found to be associated with the mitochondrial and often with the microsomal fraction of homogenized inflamed tissue when the exudate was at an alkaline *p*H. The mitochondrial-leucotaxine fraction displayed the same property as leucotaxine recovered from exudates—namely, repression by cortisone (Menkin, 1958a). The recovery of leucotaxine, which *per se* reproduces the properties of the untreated exudate—namely, that of increasing capillary permeability and of inducing leucocytic migration—does not preclude the possibility that other factors may also contribute to the phenomenon of increased capillary permeability at the incipient stage of the inflammatory reaction (Menkin, 1940a ; Miles and Wilhelm, 1955 ; Spector, 1956 ; Menkin, 1960).

Leucocytosis-promoting Factor

Let us now turn to another common denominator liberated by injured cells at the site of acute inflammation. Leucocytosis, or elevation in the number of circulating leucocytes, is often associated with various types of acute inflammatory conditions. The intravascular introduction of an exudate derived from the site of an inflammation of a dog with leucocytosis into a recipient dog induces in the latter a state of leucocytosis (Menkin, 1940b). Leucotaxine introduced into the circulation fails to induce such an effect (Menkin, 1940b). The rise in the number of circulating leucocytes is referable to a discharge of immature granulocytes from the bone-marrow. The evidence thus indicates that there is present a leucocytosis-promoting factor (L.P.F.) in an inflammatory exudate. Its presence reasonably explains in part the mechanism of leucocytosis with inflammation.

This specific factor is non-diffusible and it is thermolabile (Menkin, 1940b, 1940c). It can be purified by ammonium sulphate fractionation (Menkin, 1940c, 1956). It is absent in blood serum unless there is a concomitant acute inflammation (Menkin and Kadish, 1942). It causes specific hyperplasia of granulocytes and of megakaryocytes in the bone-marrow (Menkin, 1943a, 1956, 1958b). Chromatographic studies indicate that one is perhaps dealing with a polypeptide (Menkin, 1956, 1958b) ; though cataphoretic studies by Dillon, Cooper, and Menkin (1947) indicate that the L.P.F. is distributed in the α_1- and α_2-globulin.

Ageing the material causes a loss in biological potency and the L.P.F. becomes insoluble (Menkin, 1948a). Centrifugalization of the suspended aged material in an aqueous medium reveals, however, that the active principle is in the supernatant phase, and that this activity seems confined to a polypeptide structure. This substance recovered from canine exudate is likewise active in human beings (Menkin, 1946a, 1956). This may prove to be of clinical significance in various states of persistent leucopenia that are accompanied with only some degree of aplasia of the haematopoietic tissue in the marrow (Menkin, 1946a). Recent studies by differential centrifugation indicate that the L.P.F. is found in association with the most soluble phase or S_2 fraction of inflamed tissue when the latter is homogenized in 0.25 M sucrose (Menkin, 1958a). The L.P.F. can also be obtained as a result of cellular injury as obtained by homogenizing various tissues—for example, liver, spleen, kidney, or muscle. From the saline extract of these homogenates the L.P.F. can be recovered (Menkin, 1956).

Immunological Implications of Inflammation

I should like now to divert, in brief, attention to the immunological implications of inflammation. This, in the last analysis, is the most important aspect of this basic defence reaction. If any viable or non-viable

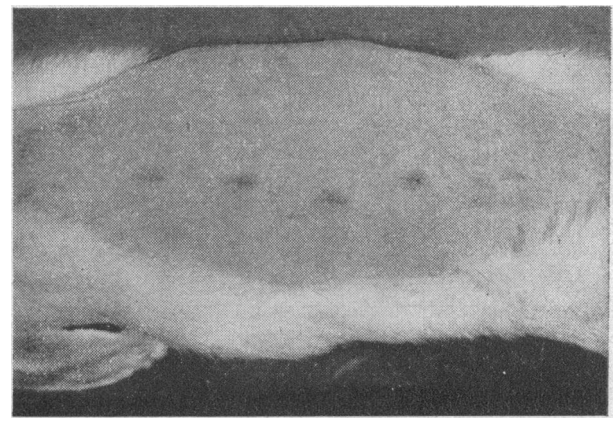

FIG. 5.—Effect of leucotaxine extracted from exudates in the incipient stage of an inflammation. Four skin areas are shown on the abdomen of a rabbit in which after the local injection of leucotaxine into each area there followed an increased capillary permeability as indicated by prompt accumulation of trypan blue from the blood into each cutaneous area. Leucotaxine was extracted from the first available exudates 4¼ to 5 hours after the injection of an irritant into the pleural cavity of a dog. This observation indicates that as soon as an exudate can be recovered, even amounting only to several millilitres, leucotaxine can already be shown to be present in such material.

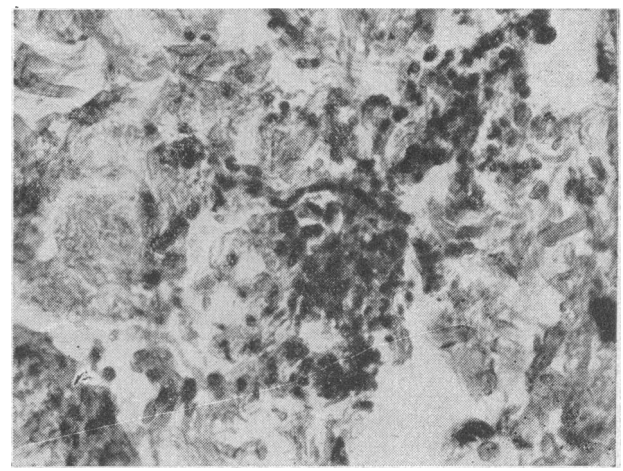

FIG. 6.—From the earliest available exudate after the injection of an irritant, leucotaxine can be extracted. In such a 4¼-hour-long inflammation extracted leucotaxine not only induces increased capillary seepage (cf. Fig. 5), but it also causes conspicuous local migration of polymorphonuclear leucocytes. (×285.)

agent is injected into an acutely inflamed area the foreign material is held fixed in the area of injury, so that it is now incapable of draining into the tributary lymphatics (Menkin, 1940a). On the other hand, when such material is injected intravenously it penetrates readily into the site of an acute inflammation (Menkin, 1940a). This is primarily due to the local increase in capillary permeability which, in the incipient stage, is largely referable to the liberation of leucotaxine by the injured cells. Leucotaxine can be identified during the first four to five hours of inflammation, when an exudate is still very scant in amount (Figs. 5 and 6). If various unrelated types of material such as a dye, a protein, graphite particles, or ferric chloride are injected into the normal peritoneal cavity they readily drain into the retrosternal lymphatic vessels. However, the induction of a peritonitis by an irritant fails after a critical interval or threshold to cause the subsequent absorption of injected substances from the inflamed peritoneal cavity as rapidly as under normal circumstances (Menkin, 1940a, 1956). This retention or fixation at the site of inflammation is referable to the deposition of a fibrinous network and to the presence of local thrombosed lymphatics (Menkin, 1931b, 1940a). There is no time to discuss all the implications of this concept.

Many years ago, by studying the relative speed and intensity with which lymphatic blockade is induced by various pyogenic micro-organisms, it was concluded that acute inflammation from an immunological point of view is the regulator of bacterial invasiveness (Menkin, 1933, 1935, 1940a). The observations presented a paradoxical situation. Staphylococci are, at least to an appreciable extent, localized owing to their marked local injurious effect that causes prompt lymphatic blockade. On the other hand, haemolytic streptococci appear to owe their penetrating or invasive properties to their relatively mild local injurious effect. This tends to maintain the patency of the lymphatics for a period of 48 hours or thereabouts. During this interval the streptococci are unobstructed in their dissemination. A concept, as a result of these observations, can be formulated as follows: $D = \frac{kt}{I}$; where D refers to dissemination, t to time, and I to the degree of induced local injury. k is a constant which may be determined by the type of irritant or by the anatomical location of the lesion (Menkin, 1940a, 1956).

Necrosin

As stated at the beginning of the presentation, inflammation is considered to be a manifestation of cellular injury in vertebrates. The basic pattern was first described at the beginning of our era by Celsus. This entails the well-known classical signs of rubor, tumor, calor, and dolor. Galen and later John Hunter added another cardinal sign—namely, loss in function. We have pointed out the presence of an additional biochemical cardinal sign—namely, proteolysis (Menkin, 1942a). It there a chemical factor concerned with the basic mechanism of the pattern of injury in inflammation ? The ultimate character of the injury may be modified by the inherent chemistry of the irritant or by the anatomical location of the lesion. In brief, a number of years ago it was found that the primary reaction seems to be referable to a toxic euglobulin liberated by injured cells at the site of inflammation, and which I have termed "necrosin" (Menkin, 1943). The α-globulins of exudates containing the L.P.F. are inactive in inducing any such characteristic lesion. The euglobulin of blood serum is likewise inactive (Menkin, 1943b, 1956).

The first morphological type of injury induced by necrosin affects the collagenous bundles. Within 10 minutes these may be swollen (Menkin, 1943b). Necrosin induces the formation of thrombi in lymphatics. The significance of lymphatic blockade in inflammation, as described above, may therefore well be referable to the liberation of necrosin by the severely injured cells. Thrombi in small blood vessels may likewise be found at the site of the acute injury (Menkin, 1943b, 1946a, 1956). Intravascular injections of necrosin frequently induce hepatic injury (Menkin, 1943b, 1946a, 1956). Repeated injections into dogs of necrosin in an endeavour to duplicate a prolonged acute process may be followed by a curious denudation of the cytoplasmic contents of liver cells (Fig. 7) (Menkin, 1946a). There is a replacement by glycogen as evidenced by appropriate cytochemical staining (Best carmine). This glycogen deposition does not seem to be referable to diet (Menkin, 1946a). Starving an animal for one or even sometimes two days yields the same abundance of glycogen in the liver of a dog repeatedly injected with necrosin. The kidney is another organ that may be frequently involved after repeated injections of this substance (Menkin, 1943b, 1946a). This may be in the form of damage to the lining epithelium of the tubules and to the irregular presence of foci of leucocytic infiltration. Recently we have found that repeated intravenous injections of necrosin or of a diffusible component from exudate often induce amyloid formation in the spleen, liver, and kidneys. This is being studied further in an endeavour to find out whether amyloid is referable to a by-product liberated by injured cells at the site of inflammation (Menkin, 1958c).

The euglobulin fraction of inflammatory exudates at an acid *p*H contains three additional chemical factors which have definite biological significance in inflammation. It was noted in the early days of our studies on this fraction of exudate that, in addition to inducing injury, leucopenia, leucocytosis, and fever were frequent accompaniments after the injections into dogs of this

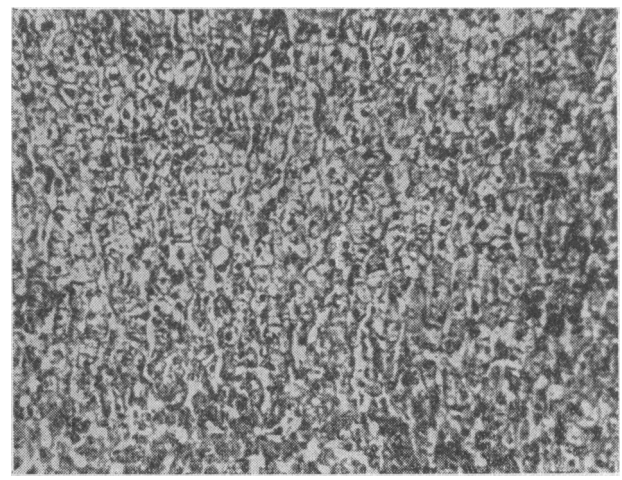

FIG. 7.—Liver of a dog that had received six intravascular injections of necrosin. Many of the hepatic cells seem denuded of their cytoplasmic contents. This appearance is referable to an abundance of glycogen deposits which are not nutritional in character. (×157.)

toxic euglobulin (Menkin, 1943b). This, as mentioned, is particularly true when the euglobulin is derived from acid exudates (Menkin, 1956).

Pyrexin

Let us examine, in brief, the primary mechanism of fever with acute inflammation. First, it is noted that the whole euglobulin fraction of exudates fails to enter readily into solution in the presence of electrolytes. This is contrary to the usual behaviour of euglobulins. Further studies have indicated that after precipitating out the euglobulin at one-third saturation with ammonium sulphate prior to dialysing out the sulphate ions, the precipitate may be treated with distilled water. Under such circumstances, a true euglobulin is found to enter into solution in the presence of the sulphate ions (Menkin, 1945). This euglobulin is necrosin. The residual insoluble fraction contains the pyrogenic factor. By this simple dissociation we have succeeded in separating necrosin from the fever-inducing factor, that has in turn been termed "pyrexin" (Menkin, 1945). At times this dissociation is not readily performed. One can then resort to merely allowing necrosin in the fluid state to stand in the refrigerator; this causes, within a few days to a few weeks, the sedimentation of pyrexin to the bottom of the container (Menkin, 1956).

Pyrexin has also been crystallized from a 50% acetic mother liquor (Menkin, 1952). The crystals appear to be rhomboid, but on slight dehydration they tend to assume a needle-like appearance. These crystals are active in inducing fever in rabbits, in contrast to the ineffectiveness of the mother liquor (Fig. 8). Necrosin contains proteolytic activity, especially when fibrinogen is utilized as a substrate (Menkin, 1946b). It is interesting to note that the incubation of necrosin is often followed by an end-product which is *per se* pyrogenic (Menkin, 1945, 1956). Conceivably, pyrexin in the euglobulin fraction of exudates may perhaps be an end-product of proteolytic activity of necrosin. Recent differential centrifugation studies indicate the presence of two pyrogenic factors at the site of inflammation and concerned in the pathogenesis of fever: one is pyrexin (thermostable) and another is thermolabile (Menkin, 1958a). It is suggested that the latter is similar to, if not identical with, the factor actively being studied by Beeson, Wood, and Bennett (Bennett and Beeson, 1953; King and Wood, 1958).

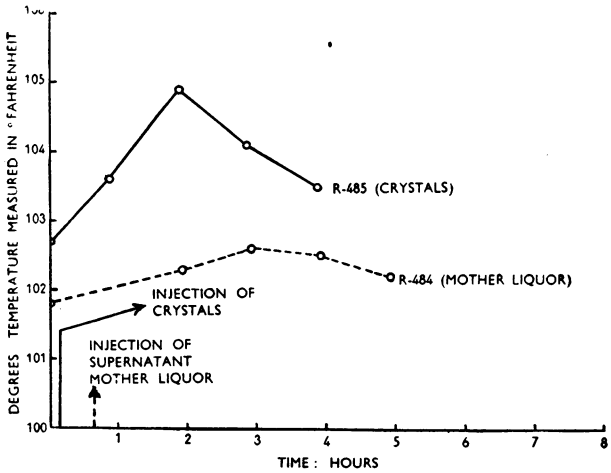

FIG. 8.—Change in temperature in a rabbit after intravenous injection of a suspension of pyrexin crystals. ——, The course of the fever after injection of the crystalline suspension; – – – –, the supernatant 50% acetic acid mother liquor fails to induce an effect similar to that obtained with the crystals. (From Menkin, 1952.)

I have given a general picture of the inflammatory reaction by illustrating how the liberation by injured cells of various chemical factors or mediators reasonably explains the biological manifestation of this important process. It can be viewed as the physical basis of infectious disease, even though inflammation in itself is a much more inclusive term, and, as pointed out above, may be caused by non-viable irritants as well. There are other factors, such as the thermostable leucocytosis factor (Menkin, 1949, 1950a) and the two leucopenic factors (Menkin, 1946c, 1948b). Some of these mediators are also recovered in the euglobulin fraction of acid exudates (Menkin, 1956). There is no time, however, to enter into a description of the other already identified factors. These have been adequately described elsewhere (Menkin, 1950b, 1956). We are beginning to shed light on the cytological precursors of these significant chemical mediators (Menkin, 1958a). It may well be that Ungar's (1953) view that histamine liberation is referable to the activity of a protease may perhaps also prove to apply to the formation of the various chemical mediators of inflammation identified by me (Menkin, 1956). These factors are biologically very specific. For instance, pyrexin induces fever, whereas the injection of the L.P.F. is followed by leucocytosis. These factors, however, are not as yet chemically pure, for even crystallization is not a criterion of purity (Shedlovsky, 1943). Their further purification looms as an important task for the future (Menkin, 1956).

As a central idea I should like to restate to you a biochemical view of inflammation—namely, that the liberation of chemical mediators by injured cells at the site of inflammation reasonably explains the diverse biological manifestations of this basic immunological process. Finally, inflammation can be regarded as an admirable system to study the biochemistry of injured cells.

The Anti-inflammatory Problem

Before terminating this discussion I wish to describe, in brief, an additional feature in the biology of inflammation. In other words, it would be well to say something about the anti-inflammatory problem. In 1940 I made the first report on the repressive effect of the adrenal cortical extract to the increased capillary permeability induced by an exudate or its contained leucotaxine (Menkin, 1940d). The same suppression was shown to occur with compound E, which eventually came to be known as cortisone (Menkine, 1942b). These were the first experimental observations on the anti-inflammatory property of cortisone.

In 1950, subsequent to the brilliant discovery by Hench, Kendall, Slocumb, and Polley (1949), at the Mayo Clinic, on the usefulness of cortisone and A.C.T.H. in arthritis, our earlier studies on this problem were resumed. We then found that neither adrenal nor cortisone repressed the increase in capillary permeability induced by an acid exudate (Menkin, 1951a). This, however, was readily suppressed by A.C.T.H. (Menkin, 1951b). There are thus at least two factors concerned in the mechanism of increased capillary permeability in inflammation: (*a*) leucotaxine, predominantly present in the initial or alkaline stage of development of the inflammatory reaction, and the activity of which is

suppressed by cortisone ; and (*b*) another factor termed "exudin," present in the later or acid stage (but independent of the *p*H) (Menkin, 1951a, 1951c). Exudin is found to be readily inhibited by A.C.T.H. but not by cortisone (Menkin, 1951a, 1951b). Crude exudin is thermolabile, non-diffusible, and has a nitrogen content of about 10%. Recent studies by differential centrifugation indicate that exudin can be isolated as a nucleopeptide, and as such is repressed by both aminotripeptidase and ribonuclease (Fig. 9) (Menkin, 1959a, 1959b). Its liberation and recovery in acid exudates reasonably explains the continuous seepage of fluid in the extracapillary spaces throughout the duration of an acute inflammation. In contrast to leucotaxine, exudin fails to induce within an hour any appreciable migration of leucocytes.

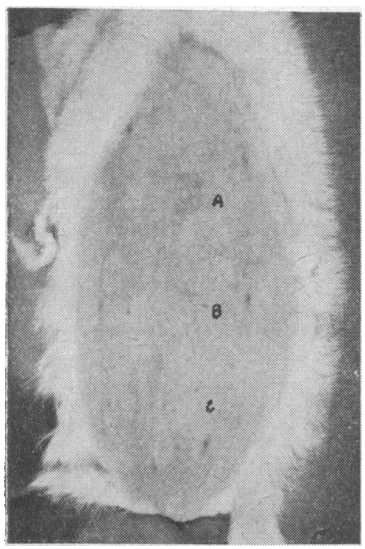

FIG. 9.—Enzymatic inactivation of purified exudin as a nucleopeptide. *Area A:* The effect of exudin, as a purified nucleopeptide, on capillary permeability—note the accumulation of trypan blue from the circulating blood into the treated cutaneous area. *Area B:* The same nucleopeptide as in Area A, but after incubation for one hour with aminotripeptidase. The effect on capillary permeability has to a large extent been eliminated. *Area C:* This nucleopeptide incubated for one hour with ribonuclease is followed by its complete inactivation. There is no dye accumulation from the circulating blood. After the injections of the material into areas A, B, and C, 5 ml. of 1% trypan blue in saline has been injected intravenously. These observations substantiate the view that exudin in the present state of purification is a nucleopeptide.

Perhaps a few words should be said about the shift in local reaction in an inflamed area. As the inflammatory reaction progresses, the exudate shifts from an alkalinity to an acidity—that is, from a *p*H of 7.2 or thereabouts to a *p*H of 6.8 or even lower. The local acidosis is referable to a glycolytic process at the site of the inflammation resulting in a lactic acid acidosis (Menkin and Warner, 1937). The polymorphonuclears are incapable of surviving this rise in hydrogen-ion concentration, and they are replaced by macrophages (Menkin, 1934, 1956). If the *p*H falls to still lower levels, all types of cells are affected, and suppuration ensues. Pus formation is virtually a function of the hydrogen-ion concentration in an acute inflammation. As stated elsewhere, these observations correlating *p*H and the cytological picture in inflammation have been confirmed in several laboratories, despite Harris's recent unwarranted assertion to the contrary (Menkin, 1956 ; Harris, 1960).

Exudin had been purified by repeated precipitation with ammonium sulphate at one-third saturation (Menkin, 1951a). It can at times be obtained in the crystalline state by maintaining it in the refrigerator after the addition of N acetic acid to the pseudoglobulin-albumin fraction of an acid exudate (Menkin, 1956). It has been obtained after dialysis of an exudate followed by treatment of the residual fraction with 95% alcohol. The resulting precipitate is discarded and the supernatant is found to contain exudin. But, as just mentioned, it has been most recently identified as a nucleopeptide (Menkin, 1959a, 1959b).

The suppressing action of A.C.T.H. on exudin seems to be a direct effect (Menkin, 1957a, 1957b). This does not controvert the classical view that A.C.T.H. acts via the adrenal cortex. In inflammation, however, there is such an extensive increase in local capillary permeability that some, and only some, A.C.T.H. injected into the blood-stream enters the site of an acute inflammation, and, once there, it can act directly on exudin (Menkin, 1956, 1957a, 1957b). In further support of this view, adrenalectomized rats display the same type of activity when injected with exudin and A.C.T.H. (Menkin, 1956, 1957a, 1957b). The question of any impurity in the commercial A.C.T.H. preparation utilized, such as vasopressin, has been eliminated by the use of Li's α-corticotrophin (Menkin, 1957a, 1957b). With his presumably pure fraction of A.C.T.H. we have obtained essentially the same inhibitory effect as with commercial A.C.T.H.

Cortisone penetrates from the systemic circulation into the site of an acute inflammation (Menkin, 1953b). This can be demonstrated by testing the exudate of a rabbit previously injected several times intravenously with cortisone, and comparing in another rabbit its reducing effect on the local passage of trypan blue with that of a control exudate. With the accumulation of the corticoids in an inflamed area there is likewise a suppression of diapedesis (Menkin, 1953b). It can also be demonstrated that repeated injections of A.C.T.H. into the circulating blood reduces the potency of exudin present in the exudate at the site of inflammation (Menkin, 1953c).

With the above information on hand, a study was undertaken in an endeavour to determine the anti-inflammatory mechanism. First, it was shown that cortisone tends to suppress cell activity in an invertebrate form (Menkin, 1953d). This was done by observing the reduction in the incidence of cell division after exposure of sea-urchin ova to corticoids prior to their fertilization. The data and interpretation on an invertebrate system cannot be readily transferred to as complicated a process as inflammation in vertebrate animals. Nevertheless, the observations on sea-urchin eggs (*Arbacia punctulata*) provided an idea. Is it possible that the anti-inflammatory mechanism is primarily referable to a suppression of cellular activity of injured cells at the site of inflammation so that now these cells are incapable of adequately forming the chemical factors that essentially constitute inflammation?

Experiments were set up to determine whether detectable or measurable amounts of active leucotaxine or of L.P.F. can be formed by the injured cells after repeated injections of hydrocortisone direct into the site of an acute inflammation (Menkin, 1954, 1956). Recovered leucotaxine was diminished in its activity (Figs. 10 and 11). The L.P.F. was likewise demonstrated to be reduced in its potency. The *in vitro* mixture of hydrocortisone (compound F) and the L.P.F. fails to reduce the activity of the L.P.F. This would indicate that the corticoid does not interact with the L.P.F., but rather that the formation of this factor by the injured cell is actually impaired (Menkin, 1954, 1956). *In conclusion,* the anti-inflammatory mechanism can be considered at a cellular level. By suppressing

the injured cell in its formation of the chemical factors or mediators involved in inflammation the inflammatory process is definitely reduced. From my point of view, it is the factors that are liberated by the injured cells which constitute inflammation. The corticoids appear to suppress the activity of the injured cells in forming these very chemical mediators.

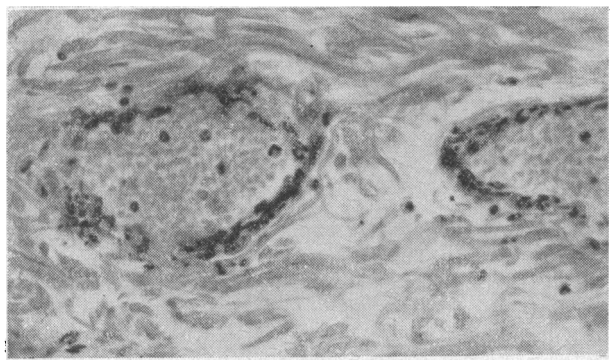

FIG. 10.—Effect of leucotaxine in inducing the margination of polymorphonuclear leucocytes about one hour subsequent to the injection of the substance in turn extracted from a sample of canine exudate. (×253.) (From Menkin, 1956.)

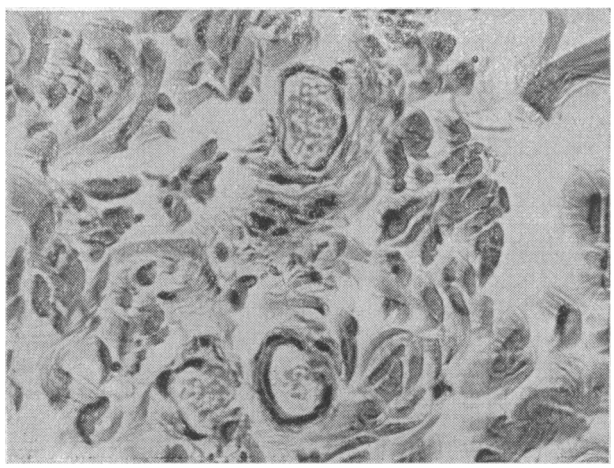

FIG. 11.—Effect on the potency of leucotaxine of two successive daily injections of hydrocortisone (compound F) into an inflamed area, amounting to a total of 40 mg. of commercial hydrocortisone. Leucotaxine was extracted from the exudate of such a treated inflamed area in the pleural cavity of a dog. Note the inability of the leucotaxine to induce leucocytic migration (cf. Fig. 10). This leucotaxine fraction, following the two hydrocortisone injections, likewise failed to increase capillary permeability. (×289.) (From Menkin, 1954.)

TABLE I.—*Sequences in the Development of the Inflammatory Reaction*

1. *Disturbance in local fluid exchange:*
   - (*a*) Initial increased capillary permeability. Referable to liberation of leucotaxine, a crystalline nitrogenous substance; suppressed by cortisone.
   - (*b*) Initial increase in lymph flow.
   - (*c*) Subsequent increased capillary permeability. Referable to exudin, in turn suppressed by A.C.T.H.
2. *Localization of irritant (fixation),* referable to lymphatic blockade by occluding thrombi and a fibrinous network at the site of severe inflammation:
   The inflammatory reaction may be regarded as the regulator of bacterial invasiveness—that is, $D=\frac{kt}{I}$, where D refers to dissemination; I, to degree of local injury; t, to time; and k is a constant.
3. *Migration of leucocytes:*
   - (*a*) Diapedesis of polymorphonuclear leucocytes, referable to liberation of leucotaxine.
   - (*b*) Cytological sequence at the site of inflammation conditioned by the local *p*H, in turn referable to a disturbance in carbohydrate metabolism; glucose formed by injured cells by deamination of proteins.
   - (*c*) Leucocytosis in the circulation, referable to two factors:
     1. Liberation of a thermolabile leucocytosis-promoting factor (L.F.F.) demonstrable in association with the pseudoglobulin fraction of exudates. The L.P.F. induces a concomitant growth of granulocytes and megakarocytes in the bone-marrow.
     2. A thermostable L.P.F. associated with the euglobulin fraction of acid exudates.
4. *The pattern of injury in inflammation,* referable to the liberation of a toxic substance in the euglobulin fraction of exudates, termed necrosin. This fraction contains proteolytic activity.
   - (*a*) Fever with inflammation, referable to liberation of pyrexin, a thermostable factor, in exudative material. Apparently a thermolabile pyrogenic factor is also present.
   - (*b*) Leucopenia with inflammation associated with the liberation of a leucopenic factor in acid exudates and with leucopenin, liberated in alkaline exudates. Pyrexin, the leucopenic factor, and the thermostable leucocytosis factor are, with necrosin components, recovered from the euglobulin fraction of usually acid exudates.
5. *Repair,* referable to the liberation of a growth-promoting factor in exudates. The factor is a diffusible component in exudative material, and appears to be consistent with being a nucleopeptide.

To conclude this lengthy discussion, an integrated summary of the principal sequences concerned in the development of an inflammation is shown in the accompanying Table I. The various mediators liberated by the injured cell are shown in Fig. 12. I hope that

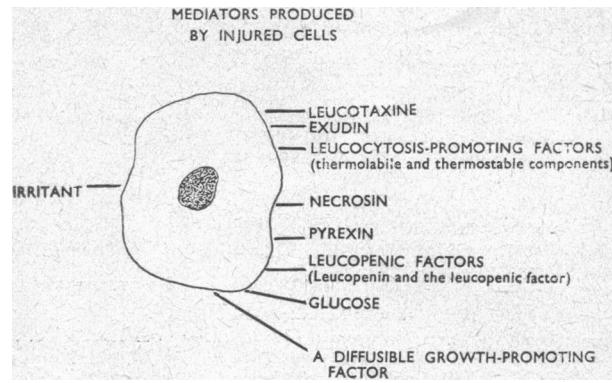

FIG. 12.—Revised and modified from a drawing in *Science*, 1947, **105**, 538, and from Menkin (1956).

interest in probing further into the biochemistry of the injured cell may have been aroused by this panoramic discussion of inflammation, a phenomenon that has always been considered of basic importance in the study of pathology.

Summary

Inflammation is a manifestation of severe cell injury in vertebrates. The inflammatory reaction is initiated by disturbance in local fluid exchange. The most important alteration is a local increase in capillary permeability. This is brought about in large part by the liberation of leucotaxine by injured cells at the site of inflammation. This is a diffusible substance which appears to be a polypeptide. It has also the property of inducing the migration of leucocytes through the endothelial wall. Another mediator present in inflammatory exudates is termed the leucocytosis-promoting factor (L.P.F.). This factor, in addition to the

thermostable leucocytosis factor, provides a reasonable explanation for the mechanism of leucocytosis in inflammation.

The immunological implication of inflammation is discussed, and it is pointed out that the role of inflammation is that of a regulator of bacterial invasiveness. The pattern of injury in inflammation is ascribed to the liberation of a toxic euglobulin termed necrosin. Fever is shown to be due to two factors—namely, a thermostable component termed pyrexin, and a thermolabile factor.

The studies on the anti-inflammatory problem, first described by me in 1940 and 1942, are summarized ; and it is shown that the mechanism of action is referable to a suppression of cellular activity. The injured cell is incapable of forming the mediators of inflammation as readily as under normal circumstances.

A summary of the development of the inflammatory reaction as well as concluding remarks on the significance of the biochemistry of inflammation are pointed out.

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NEONATAL HYPERBILIRUBINAEMIA*

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[WITH SPECIAL PLATE]

Jaundice is commonly observed in newborn infants. It is probable that 40% of infants have a serum bilirubin level of at least 4 mg. per 100 ml. during the first week of life. For this reason the term "physiological jaundice" has crept into use. This is an unfortunate description, as it tends to minimize the risk to the patient. Hyperbilirubinaemia can be dangerous or even lethal in certain patients. The danger lies in the development of cerebral nuclear jaundice (kernicterus). This may either kill the infant or leave him with permanently damaged areas in the brain. It must be stressed, therefore, that in certain circumstances jaundice in the newborn period is to be regarded as pathological and of serious import for the future of the child. Fortunately, hyperbilirubinaemia of this magnitude is not common in the absence of an actual disease process such as haemolytic disease of the newborn. The danger is that the necessary corrective treatment will be instituted too late if jaundice in otherwise normal infants is regarded as being entirely physiological.

The nature of the bile pigment responsible for the hyperbilirubinaemia is of diagnostic and prognostic significance. Ehrlich's method for demonstrating the presence of bilirubin in urine was adapted by van den Bergh and Snapper (1913) for the estimation of bilirubin in plasma It was later found (van den Bergh and Muller, 1916) that alcohol was not an essential for the development of the colour reaction when bile was used or if the patient was suffering from obstructive jaundice. In patients suffering from haemolytic jaundice alcohol was still necessary, and the reaction was called "indirect." The direct and indirect forms of the van den Bergh reaction have been used for many years in the diagnosis of obstructive and non-obstructive jaundice. It was not clear, however, whether two separate bile

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